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Effects of dry period length on milk yield and content and metabolic status of high-producing dairy cows under heat stress

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Abstract

The objective of the present study was to investigate the effects of shortening dry period (DP) on milk yield, milk composition, and blood parameters in cows under heat stress. For this purpose, three DP groups were considered (30, 45, and 60 days), and 14 heat-stressed high-producing dairy cows (7 primiparous and 7 multiparous) were assigned to each DP group. The results showed that shortening the DP (from 60 to 30 days) decreased a 305-day milk yield in subsequent lactation. The difference in milk yield between 30- and 60-day DP groups was significant in the second 100 days of lactation. Accounting for additional milk yield before calving completely compensate for the loss of milk production resulting from shortening the DP. Milk protein, lactose, and solids non-fat percentages were significantly lower for cows with a 60-day DP compared to cows with a 45- or 30-day DP. Serum BHBA and urea concentrations were significantly higher, and serum glucose concentration was significantly lower for cows with a 60-day DP compared to cows with a 45- or 30-day DP. Serum such as 0-day DP compared to cows with a 30-day DP. In conclusion, results showed that shortening the DP improved the metabolic status of heat-stressed transition dairy cows without decreasing total milk yield.

Keywords Heat stress · Dry period · Early lactation · Milk composition · Blood parameters

Introduction

Modern dairy cattle are more susceptible to changing climatic conditions due to intensive selection for high milk production (Sammad et al. 2020). High milk yield requires a high metabolic activity and thus generates more metabolic heat. Elevated internal heat production coupled with increasing environmental temperature leads to heat stress (Sammad et al. 2020; Brown et al. 2016; and West et al. 2003). Heat

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stress is one of the most important challenges facing the dairy industry today (Polsky and von Keyserlingk 2017). It has a negative impact on a variety of performance parameters such as milk yield, reproduction, and growth (Sammad et al. 2020).

In early lactation, the nutrient requirements of dairy cattle usually exceed feed intake which may result in different degrees of negative energy balance (NEB) (Butler 2003). Under heat stress conditions, cows may go further into NEB in postpartum period which may result in decreased concentrations of plasma insulin, IGF-I, and glucose. These factors are necessary for normal folliculogenesis, and their dysregulation may lead to reproductive disorders (De Rensis and Scaramuzzi 2003). Mobilization of body fat reserves is an adaptive response to NEB in dairy cattle (White 2015; Bünemann et al. 2019). Nonesterified fatty acids (NEFA) release from adipose tissue in the postpartum period may exceed the oxidation capacity of the liver. Excessive NEFA may be incompletely oxidized by the liver to ketone bodies or reesterified to triglycerides, potentially leading to ketosis or fatty liver (Adewuyi et al. 2005). The severity of NEB, which is characterized by the degree of increase in plasma concentrations of NEFA and beta-hydroxybutyric acid (BHBA) and the degree of decrease in glucose plasma concentration, may also contribute to the immunosuppression in transition cows (Moyes et al. 2009).

A dry period (DP) of approximately 8 weeks has been recommended for dairy cattle. The conventional DP facilitates the involution of mammary gland and the replacement of senescent mammary epithelial cells with active cells (Kok et al. 2017; Capuco et al. 1997) and has been thought to maximize milk yield in the subsequent lactation. Decreasing the DP is associated with reduction in milk production, after calving. Some studies proposed that decreasing or omitting the DP can improve the energy balance, fertility, and health of dairy cattle (Andersen et al. 2005; Gümen et al. 2005; Chen et al. 2015; Kok et al. 2017). Watters et al. (2008) showed that incidences of metritis, retained placenta, and displaced abomasum were not affected by DP length. Most studies indicated that shortening the DP length improved reproductive performance but the effect of shortening DP on udder health is ambiguous (Kok et al. 2019). van Hoeij et al. (2016) concluded that DP length did not affect the occurrence of clinical mastitis but cows with no DP had greater somatic cell counts (SCC) compared with cows with a 30-day or 60-day DP. Annen et al. (2004) indicated that shortening the DP length had no significant effect on SCC in subsequent lactation. Previous studies indicated that days open (Gümen et al. 2005) and the mean interval from calving to first ovulation were longer (Watters et al. 2009) in cows with a long DP than in cows with a short DP. Additionally, cows with a short or no DP had less NEB compared with cows with a traditional DP of 8 weeks (Rastani et al. 2005). Moreover, a meta-analysis indicated that shortening the DP length tends to decrease the risk of ketosis in the next lactation (van Knegsel et al. 2013). In the present study, the effects of shortening DP on milk yield, milk composition, and metabolic status of high-producing dairy cows under heat stress were investigated.

Materials and methods

Cows and treatments

This study was conducted in compliance with the guidelines approved by University of Mohaghegh Ardabili according to the guidelines of the Animal Care and Use Committee. In this study, 42 high-producing dairy cows (21 primiparous and 21 multiparous) from a commercial Holstein dairy herd in Ardabil province in Iran were assigned to three dry period groups (30, 45, and 60 days) (completely randomized design). Cows included in the present study showed no clinical signs of mastitis.

Milk yield and sampling

Cows were milked three times a day, and sum of these records were considered the daily record for each cow. Milk sampling was performed weekly from parturition until 4 weeks postpartum. Milk samples were treated with preservative and sent to laboratory for analysis. The samples were analyzed for fat, protein, lactose, solids, and solids non-fat.

Fat-corrected milk (FCM), energy-corrected milk (ECM), and solids-corrected milk (SCM) were calculated using the following formulas (NRC 2001):

 $\begin{aligned} 3.5\% FCM &= 0.432 \times \text{milk yield} + 16.23 \times \text{fat yield} \\ ECM &= 12.82 \times \text{fat yield} + 7.13 \times \text{protein yield} + 0.323 \times \text{milk yield} \\ SCM &= \text{milk yield} \times \left[(12.24 \times \text{fat}\% \times 0.01) + (7.1 \times \text{protein}\% \times 0.01) + (6.35 \times \text{lactose}\% \times 0.01) - 0.0345 \right] \end{aligned}$

Calculation of milk yield in different parts of lactation

Additional milk yield during the 60 days before calving, the 305-day milk yield and milk yield during three parts of lactation (0–100, 100–200, and 200–300 days in milk) were estimated, using incomplete gamma function (Wood 1967). The function is as follows (Eq. 1): $Y_t = at^b e^{-ct}$ where variable *t* is days in milk (DIM), Y_t is the milk yield (kg/d) at DIM *t*, *e* is the Neper number, *a* represents yield at the beginning of lactation, and *b* and *c* are the factors associated with inclining and declining slopes of the lactation curves, respectively. Wood function was transformed logarithmically into a linear form and fitted to test day milk records of each cow. The linear model was as follows (Eq. 2): $\ln(y_t) = \ln(a) + b\ln(t) - ct$.

After estimation of lactation curve parameters for each cow, milk yield during each part of lactation was estimated by integrating Eq. 1. A simple program (written in Visual basic 6) was used for the above calculations.

The daily maximum THI (temperature humidity index) was calculated using the following formula (Vitali et al. 2009):

$$\text{THI} = (1.8 \times \text{AT} + 32) - (0.55 - 0.55 \times \text{RH})$$

 $\times [(1.8 \times AT + 32) - 58]$

In this formula, AT is the ambient temperature (°C), and RH is the relative humidity as a fraction of the unit.

The daily maximum THI was 88.55 (± 2.95) on average, during the present study.

Blood sampling and analysis

Blood samples were taken four times: one week before parturition, 10, 20, and 30 days after parturition. The sera were separated by centrifugation (at $3000 \times g$ for 15 min), stored at -20 °C until assayed, and analyzed for BHBA, NEFA, glucose, cholesterol, and urea. Serum cholesterol, glucose, and urea concentrations were determined by an autoanalyzer (Abbott Alcyon 300, Abbott Diagnostics, Lake Forest, IL) using commercial kits (Pars Azmoon Co., Tehran, Iran) according to the manufacturer's instructions. The concentrations of serum BHBA and NEFA were also measured using an autoanalyzer and commercial colorimetric kits (Randox Laboratories Ltd., Ardmore, UK).

Statistical analysis

Statistical analyses were carried out applying the MIXED procedure of SAS software (SAS Institute Inc., Cary, NC). Variables repeated in time were analyzed as repeated measurements. The effect of cow in each treatment was considered a subject. Lactation number, treatments (DP groups), and the time of recording (1, 2, 3...) were fixed effects. Time × treatment interaction was also included in the model. For statistical analysis of blood parameters, analysis of the first blood sample (1 week before parturition), and statistical analysis of milk yield, 305-day milk production in previous lactation was considered covariate.

Results and discussion

Postpartum milk yield and additional yield before calving

There was a tendency for cows in the 30-day DP group to have a lower milk yield (32.01) compared with cows in the 60-day DP group (34.62 kg) in the first month of lactation (P < 0.09). Mean daily milk yield was also lower for cows with a 45-day DP (32.66 kg) than for cows with a 60-day DP. The difference between daily milk yield of cows with 30- and 60-day DP was significant, in the first week of lactation (P < 0.05) (Table 1). Gulay et al. (2003) concluded that there is no significant difference in milk yield between cows with 30- and 60-day DP during the first 10 weeks of lactation (38.4 vs. 38.7 kg per day, respectively). Rastani et al. (2005) reported that milk yield was lower for cows with 28-day DP compared to cows with 56-day DP in both primiparous and multiparous cows. Santschi et al. (2011) reported that shortening the DP length reduced milk yield of cows in parity 2 but had no effect on milk yield of older cows. Kok et al. (2017) reported that 305-day milk yield decreased about 5.6 kg per day after one omission of the DP (after a standard previous DP).

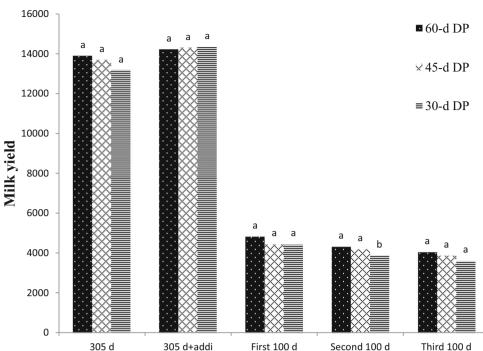
Cows with 60-day DP produced the highest milk yield in all parts of lactation however, the difference between DP groups in the first 100 days of lactation was not significant but the difference between 30- and 60-day DP groups was significant in the second 100 days of lactation. There was also a tendency for cows in the 30-day DP group to have a lower milk yield compared with cows in the 60-day DP group in the third 100 days of lactation (P < 0.1). The results also showed that accounting for additional milk yield before calving completely compensate for the loss of milk production resulting from shortening the DP (from 60 to 30 days or 45 days) (Fig. 1).

Shortening or omitting DP shifts milk production from the postcalving period to the period before calving (van Knegsel et al. 2014; Grummer et al. 2010). Different studies indicated that shortening the DP (from 60 to 30 days) reduced milk yield after calving by about 2-11% (Annen et al. 2004; Rastani et al. 2005). Kok et al. (2016) showed that cows with a short or no DP had lower days open (18 days and 25 days, respectively) compared with cows with a conventional DP. They concluded that taking into account these differences in calving interval and additional milk yield before calving decreased milk losses for cows with a short or no DP. Schlamberger et al. (2010) concluded that by taking into account the additional milk yield before calving, total loss in 305-day milk yield decreased from approximately 16 to 6%, when cows with no DP were compared with cows with a 56-day DP. In the study of van Knegsel et al. (2014), the additional milk

 Table 1
 Least square means (±LSM) of daily milk yield (kg) during the first month of lactation in three dry period (DP) groups

Time of lactation	DP length			<i>P</i> value			
	30	45	60	30 vs.45-day DP	30 vs. 60-day DP	45 vs. 60-day DP	
First month	32.01±6.68	32.66±6.51	34.62±6.59	0.67	0.09	0.22	
First week	$19.57 {\pm} 7.00$	21.48 ± 6.78	24.27 ± 6.90	0.35	0.02	0.18	
Second week	24.83 ± 7.34	24.41 ± 7.10	27.75 ± 7.24	0.83	0.16	0.12	
Third week	40.86 ± 9.65	40.01 ± 9.30	44.01±9.43	0.70	0.16	0.08	
Fourth week	38.65±9.21	$40.03 {\pm} 8.98$	$38.89 {\pm} 8.79$	0.46	0.90	0.55	

Fig. 1 Least square means (±LSM) of total milk yield in different parts of lactation; 305 d = 305 days milk vield. 305 d + addi = 305 days milk yield + additional milk yield during the 60 days before calving, first 100 d = milk yield from calving to 100 DIM, second 100 d = milk yield from 100 to 200 DIM, and third 100 d = milk yield from 200 to 300 DIM



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yield did not completely compensate for the loss of milk yield resulting from omitting or shortening the DP. They concluded that higher concentrations of milk compositions fully compensated for the loss of milk production for multiparous cows but not for primiparous cows. Shoshani et al. (2014) indicated that adding the additional milk production before calving to the 305-day milk yield postcalving leads to higher milk yield for cows with 40-day DP compared to cows with conventional DP. Their results were in line with the results of the current study. Kok et al. (2017) found that cows with no DP for consecutive lactations of 305-days, had higher milk production and lower additional milk yield of +560 kg and -172 kg of fat-protein-corrected milk, respectively compared with cows with no DP for the first time. This could decrease the effect of omitting the DP on improvement of the energy balance for cows that receive no DP for the second time (or more).

Milk composition

Milk protein (%), lactose (%), and solids non-fat (%) were significantly lower for cows with 60-day DP compared with cows with 45- and 30-day DP. There was no difference in milk composition between cows with 30- and 45-day DP (Table 2). Rastani et al. (2005) reported increased milk protein percentage, in cows with 28-day DP compared with cows with a traditional DP which is in accordance with our results. They also concluded that milk protein yield from cows with short or no DP was not significantly different from cows with a traditional DP. Rémond et al. (1992) reported increased milk protein yield for cows without a dry period (0-day DP) compared with cows with a 60-day DP. van Knegsel et al. (2013) explained that shortening or omitting the DP of Holstein dairy cattle would decrease milk production, increase milk protein percentage, and tend to reduce the probability of ketosis in subsequent lactation. In the other study of van Knegsel et al. (2014) indicated that shortening the DP length (from 60 to 30 days) increased milk protein percentage and reduced total milk yields. However, greater milk solid not fat and milk lactose percentages for cows with a short DP (35 days) compared to cows with a traditional DP (60 days), which is in accordance with our results (Watters et al. 2008) (Table 3).

There was no significant difference in milk fat percentage among the dry period (DP) groups. Gulay et al. (2003) reported no difference in milk fat percentage and milk fat yield postpartum for cows with a 30- vs. 60-day DP. Rastani et al. (2005) reported a tendency for increased (P < 0.1) milk fat percentage in the subsequent lactation for cows with a 30-day DP compared with cows with a 60-day DP. Their results also indicated that there was no difference in milk fat yield between cows that received a 30- vs. 60-day DP. Kuhn et al. (2006) concluded that DP lengths of less than 20 days would result in substantial losses in fat and protein yield in the next lactation. Rémond et al. (1992) indicated that there is no significant difference in milk fat yield from cows with a 60-day DP compared to cows with no DP.

Blood metabolite concentrations

Serum BHBA and urea concentrations were higher (P < 0.05), and serum glucose was lower (P < 0.05) for cows with a 60day DP than in cows with a 30-day DP. Serum glucose was

Table 2 Least square means (±LSM) of milk composition, FCM, ECM, and SCM during the first month of lactation in three dry period (DP) groups

Variable	DP length			<i>P</i> value			
	30	45	60	30 vs.45-day DP	30 vs. 60-day DP	45 vs. 60-day DP	
Fat (%)	3.49±1.05	3.35±1.01	3.51±1.00	0.58	0.96	0.58	
Protein (%)	3.41±0.39	$3.33 {\pm} 0.38$	3.12 ± 0.38	0.4	0.006	0.04	
Lactose (%)	4.81 ± 0.22	4.85 ± 0.22	4.62 ± 0.21	0.39	0.002	0.0002	
Solids (%)	13.21 ± 1.7	13.01±1.6	12.95 ± 1.6	0.59	0.52	0.88	
Solids non-fat (%)	$9.28 {\pm} 0.59$	9.21±0.57	$8.72 {\pm} 0.57$	0.66	0.0008	0.003	
FCM	42.4±14.2	41.1±13.7	46.1±13.6	0.66	0.29	0.15	
ECM	42.7±12.54	41.3±12.05	45.4±12.01	0.62	0.37	0.19	
SCM	40.6±12.08	39.13±11.61	42.3±11.59	0.57	0.58	0.29	

FCM mean daily fat-corrected milk production, ECM mean daily energy-corrected milk production (ECM), SCM mean daily solids-corrected milk production

also lower (P < 0.05) for cows with a 60-day DP compared to cows with a 45-day DP. Cows in the 60-day DP group tended to have higher serum BHBA and urea compared to cows in the 45-day DP group. There was also a tendency for cows in the 45-day DP group to have higher serum urea and lower serum glucose compared to cows in the 30-day DP group (Table 3).

Serum concentrations of nonesterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) are used as indicators of negative energy balance (NEB). The NEB induces lipid mobilization and lipolysis in dairy cows. Fatty acids released from adipose tissue lipolysis circulate as NEFA. The concentration of NEFA in blood indicates the degree of adipose tissue mobilization, whereas BHBA concentration reflects the completeness of fat oxidation in the liver (Leblanc 2010; Roberts et al. 2012). Since BHBA is the main circulating ketone body in ruminants, blood BHBA concentration is a gold standard diagnostic test for hyperketonemia (Oetzel 2004). Different researches have studied the BHBA concentration threshold in which future health and production is impaired.

LeBlanc et al. (2005) recommended that the risk of displaced abomasum would increase at serum BHBA concentrations above 1.2 mmol/L, during the early lactation period. Suthar et al. (2013) showed that cows with serum BHBA concentrations of ≥ 1.1 , ≥ 1.4 , and ≥ 1.7 mmol/L had 10.5, 1.7, and 6.9 times greater odds of developing clinical ketosis, metritis, and displaced abomasum, respectively, compared with cows with lower serum BHBA concentrations. Duffield et al. (2009) proposed a serum concentration of 1.2 mmol/L as a threshold, above which the disease incidence rate and production are affected. Most published studies recommend a serum BHBA threshold of 1.2 mmol/L (Santschi et al. 2016). Serum concentration of BHBA for cows with a 60-day DP (1.34 mmol/L) was higher than threshold (1.2 mmol/L), but it was lower for cows with 45-day (0.92 mmol/L) and 30-day (0.81 mmol/L) DP.

In the study of Jolicoeur et al. (2014), blood BHBA level was significantly lower for cows with short DP (35-day DP) compared to cows with conventional DP, during the early lactation period. Rastani et al. (2005) concluded that shortening the DP (from 56 to 28 days) did not affect serum concentration of NEFA and BHBA postpartum, significantly. Chen et al. (2015) concluded that DP length had no effect on serum BHBA concentration. Andersen et al. (2005) reported that omitting the dry period decreased the concentration of serum

Variable	DP length			<i>P</i> value		
	30	45	60	30 vs.45-day DP	30 vs. 60-day DP	45 vs. 60-day DP
BHBA (mmol/L)	0.81±0.17	0.92±0.22	1.34±0.17	0.67	0.04	0.08
NEFA (mmol/L)	$0.83 {\pm} 0.15$	$0.94 {\pm} 0.20$	0.93 ± 0.15	0.62	0.62	0.97
Glucose (mg/dl)	51.05 ± 1.9	45.23±2.4	37.33±1.9	0.06	0.0003	0.0083
Cholesterol (mg/dl)	144.3 ± 10.6	145.3±13.6	147.7±10.9	0.95	0.82	0.87
Urea (mg/dl)	30.96±1.3	34.19±1.6	37.74±1.3	0.1	0.003	0.07

Table 3 Least square of means (±LSM) of serum metabolite concentrations during the first month of lactation in three dry period (DP) groups

BHBA and increased the concentrations of glucose, indicating an improved metabolic status in early lactation for cows with a 0-day DP. The effect of shortening the DP to 45 or 30 days was not studied in their research. Klusmeyer et al. (2009) showed that shortening the DP (from 60 to 32 days), of cows supplemented with recombinant bovine somatotropin, decreased serum concentration of BHBA in early lactation.

The controversy between the results of the present research and some previously mentioned studies (Rastani et al. 2005; Chen et al. 2015) could be due to the impact of heat stress in our study. In the present study, cows were under heat stress in early lactation. Basiricò et al. (2011) concluded that summercalving cows had lower levels of glucose and higher levels of BHBA compared to spring-calving cows. Results of the present study showed that cows with a 60-day DP had significantly lower levels of glucose than cows with a 30- and 45day DP. Pezeshki et al. (2007) concluded that shortening the DP from 56 to 28 days had no effect on concentrations of serum glucose during early lactation. In Rastani et al. (2005) there was a tendency for cows with a 28-day DP to have a greater (P < 0.15) plasma glucose concentration compared to cows with a 56-day DP.

The energy status of dairy cows during early lactation critically affects metabolic parameters and reproductive performance (Butler and Smith 1989; Jorritsma et al. 2003 and Mellouk et al. 2019). The NEB is associated with reproductive disorders such as postpartum metritis and endometritis (Sheldon et al. 2018). Monitoring glucose in early lactation could be used to detect cows at risk for infertility (Garverick et al. 2013). One study indicated that cows that became pregnant at first artificial insemination had greater plasma glucose during the first 30 days of lactation when compared to cows that failed to conceive but there was no relationship between pregnancy at first artificial insemination and plasma glucose at 30–60 days postpartum (Green et al. 2012).

The plasma concentration of urea for cows with a 60-day DP was higher (P < 0.05) than that of cows with a 30-day DP. Chen et al. (2015) indicated that shortening the DP (from 60 to 30 days) did not affect plasma glucose and urea concentrations. However, Jolicoeur et al. (2014) showed that glucose and urea concentrations were not affected by DP length, but in the present study, glucose and urea concentrations were significantly different, between DP groups. The difference between the results of the present study and some previous studies also could be due to the presence of heat stress in the current study. Urea is a product of protein catabolism. Energy deficiency seems to stimulate catabolism of amino acids from tissue proteins, resulting in increased urea production (Bell 1995). Furthermore, impairment in liver function during NEB may lead to decreased metabolic clearance of urea (O'Callahan et al. 2001; Cheng et al. 2015).

Wathes et al. (2007) indicated that urea concentration could be a useful predictor of fertility. Gao et al. (2017) found that heat stress reduced plasma concentration of free amino acids (17.1%) and glucose (8%) and also increased milk, urine, and blood urea nitrogen (24.5, 243, and 17.2%, respectively). They concluded that heat stress may lead to increase in systemic amino acid utilization. Transition period is the most critical phase of the production cycle of dairy cows. Heat stress is one of the main factors negatively affecting health, fertility, and production of dairy cattle. Therefore, the metabolic status of transition dairy cows may be worsened in heat stress (Basiricò et al. 2011). Kok et al. (2017) explained that a short or no DP may reduce the incidence of involuntary culling and replacement costs due to improved fertility.

Conclusion

Decreasing the dry period to 30 days may improve the metabolic and energy status of transition dairy cows without decreasing total milk yield, when cows are under heat stress during early lactation.

Authors' contributions AB and VV conceived and designed research. AB and MA conducted experiments. MA and HK contributed new reagents or analytical tools. RS, NH, and CG analyzed data. AB, VV, and MA wrote the manuscript. AZM edited and revised the manuscript as well as prepared it for journal submission. All authors read and approved the manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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